HHS Public Access

Author manuscript

Curr Opin Allergy Clin Immunol. Author manuscript; available in PMC 2018 March 15.

Published in final edited form as:

Curr Opin Allergy Clin Immunol. 2012 April ; 12(2): 102–110. doi:10.1097/ACI.0b013e3283511396.

The role of lymphocyte proliferation tests in assessing occupational sensitization and disease

Stella E. Hines, MD, MSPH[1,2], **Karin Pacheco, MD, MSPH**[3,4], and **Lisa A. Maier, MD, MSPH**[3,4,5]

[1]Occupational Health Program-Division of General Internal Medicine, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD

[2]Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD

[3]Division of Environmental and Occupational Health Sciences, Department of Medicine, National Jewish Health, Denver, CO

[4]Department of Environmental and Occupational Health, Colorado School of Public Health, Denver, CO

[5]Division of Pulmonary Sciences and Critical Care Medicine Department of Medicine, School of Medicine, University of Colorado-Denver Denver, CO

Abstract

Purpose of Review—Lymphocyte proliferation testing (LPT) is used in diagnosing occupationally-acquired delayed-type hypersensitivity. It has been used in beryllium-health effects, and it role is expanding in metal allergy. It may find application in diagnosis of other sensitizers.

Recent findings—Use of the beryllium LPT (BeLPT) in medical surveillance identifies beryllium sensitization at low exposure with chronic beryllium disease (CBD) that leads to physiologic impairment and need for immunosuppressive medications. New studies indicate that both beryllium exposure and genetic variation are associated with increased risk of CBD. Borderline positive BeLPTs warrant inclusion into diagnostic algorithms. Furthermore, use of LPTs to diagnose metal allergy is being proposed in diagnosis of chromium allergy and hypersensitivity to surgical implants. New occupational sensitizers continue to be identified including metalworking fluids, the sterilizing agent ortho-phthalaldehyde and the solvent parachlorobenzotrifluoride. Use of LPT in occupational surveillance to these agents, and other known sensitizers may play expanding roles.

Contact for corresponding author: Stella E. Hines, MD, MSPH, Assistant Professor, The University of Maryland School of Medicine, Department of Medicine, Occupational Health Program and Division of Pulmonary & Critical Care Medicine, 11 S. Paca Street, Second Floor, Baltimore, MD 21201, phone 410-706-7464; fax 410-706-4078, shines@medicine.umaryland.edu.

Summary—Lymphocyte proliferation testing serves a valuable role in diagnosing occupational sensitization, as demonstrated with beryllium-health effects, as cases continue to be found at low exposure levels. The use of LPTs in diagnosing contact allergy is expanding, and new applications may be identified in human and animal studies.

Keywords

lymphocyte proliferation test; beryllium; chronic beryllium disease; contact allergy; sensitization; occupational surveillance

Introduction

Numerous occupations and environments expose workers to allergens/antigens that can cause sensitization and disease. Traditional methods of determining sensitization include prick skin testing, intradermal skin testing, IgE radioallergosorbent tests, and patch testing, although each of these methods has their drawbacks. The lymphocyte proliferation test (LPT), measuring cell-mediated T cell responses to specific antigens, serves as a useful tool in the clinical evaluation and medical surveillance of exposed workers. Use of LPTs is effective in the early identification of disease risk, and in secondary prevention of disease. This review will examine the utility of the LPT in medical surveillance and focus on recent updates in the use of LPTs to identify responses to occupational sensitizers.

Background

Lymphocyte proliferation testing assesses delayed type hypersensitivity reactions *in vitro* wherein an antigen interacts with an antigen presenting cell (APC), which then activates antigen specific T cells to proliferate. In vivo this process results in a cascade of immunologic events, cytokine release and disease [1]. Lymphocyte proliferation testing has been used to assess hypersensitivity to various materials, but most prolifically with beryllium.

As lymphocyte proliferation testing has evolved, so has LPT methodology as is evidenced by the changes in the analysis and interpretation of the beryllium LPT [2–4]. In general, cells are grown in culture for several days with and without antigen at varying concentrations. After a specified time, tritiated thymidine, a radiolabeled DNA precursor, is added to cell culture. Proliferation is assessed by the degree of cellular uptake of radiolabeled thymidine. Results are expressed as a stimulation index (SI), a ratio of radiolabeled thymidine uptake in the stimulated, antigen-exposed cells compared to uptake in the unstimulated cells [1]. A positive test is generally identified by two or more SIs exceeding a specific threshold of abnormal. In the case of the BeLPT, the threshold of abnormal typically either exceeds a specific cut-point (eg in some labs an SI ≥ 3.0) or relies on statistical determination of a mean peak SI among unexposed, nonsensitized subjects [3,4]. With regards to BeLPTs, a test may be interpreted as normal, if no SI is above the cut off level. When the SI of only one of the concentrations of beryllium is elevated above the cut off, the test is considered “borderline” (BL). Two or more elevated SI’s is considered abnormal.

The animal model correlate of the LPT, the Local Lymph Node Assay (LLNA), relies on dermal exposure as a route leading to systemic sensitization [5]. Briefly, an antigen is topically applied to an animal's ear – typically a mouse or rabbit- for several days, followed by ear thickness assessments to evaluate irritancy. Subsequently, the animal is injected with tritiated thymidine and the draining lymph nodes and their lymphocytes are harvested. The exposed animal's lymphocyte suspensions are evaluated for incorporation of tritiated thymidine, and compared to unexposed animals to determine stimulation indices. An SI is calculated for the proliferation of lymphocytes in the stimulated mice compared to control mice [6,7]. Identification of sensitizers via LLNA targets exposures that may result in sensitization and warrant further investigation in human populations.

Review of LPT uses to assess biologic responses to various agents

LPTs have been utilized in occupational medical surveillance and clinical diagnosis of diseases induced by several agents to be reviewed here.

Beryllium

To date, the LPT has had greatest clinical application in its use screening workers for beryllium related health effects. Exposure to beryllium can lead to beryllium sensitization (BeS) and chronic beryllium disease (CBD) in susceptible individuals. BeS is the immunological precursor to CBD and is diagnosed by demonstration of a beryllium stimulated immune response via the BeLPT. CBD manifests as a granulomatous lung disease and is generally diagnosed based on the demonstration of sensitization to Be manifest either by lymphocyte proliferation responses of the peripheral blood or bronchoalveolar lavage (BAL) cells in response to Be stimulation, and the presence of non-caseating granulomas on lung biopsy [1]. To determine if an individual has CBD, a bronchoscopy is usually required as the BeLPT does not differentiate between CBD and BeS. The diagnostic criteria noted above emerged following epidemiologic investigations of the use of the BeLPT [8–12]. Most recent studies demonstrate that the positive predictive values (PPV) of two abnormal tests to diagnose BeS range from 96.8% to 99.7% [13–15]. Six to eight percent of BeS patients progress to CBD annually [16]; although the clinical severity of CBD detected by workplace surveillance has varied [17].

Updates on Beryllium Sensitization—Studies by Stange and Middleton provided the most recent probability characterizations for one versus two positive BeLPTs to diagnose BeS [13–15]. The Middleton algorithms to diagnose BeS include the use of BL tests, with the presence of one abnormal (AB) plus one borderline (1 AB + 1 BL), but some diagnostic algorithms and compensation directives do not recognize the value of the borderline test as a measure of abnormality [2,14,15]. Because the predictive value of (1 AB + 1 BL) approaches that of 2 abnormals (AB) over a range of BeS prevalences, cases of CBD could be missed if evaluations are limited to workers with at least 2 AB results [14]. In most surveillance programs, an initial AB or BL is followed by a "split" sample sent to two labs, ultimately leading to 3 tests. Using data from the Stange 2004 study, Middleton demonstrated that the post-test probabilities for the 3-result combinations possible of suggesting sensitization at 2% prevalence were: 3 AB (100%), 2 AB+1BL (100%); 1 AB + 2

BL (99%); 2 AB + 1 NL (95%); 3 BL (91%); 1 AB + 1 BL + 1 NL (72%) [13,18]. These results suggest that BL results do have meaning and that the combination of 3 BL has a sufficiently high predictive value to refer patients for diagnostic evaluation.

Updates on BeLPT in diagnosis of CBD or CBD Severity—Critics of the LPT have questioned its significance in workplace surveillance, arguing that it does not detect clinically severe CBD. However, longitudinal follow-up of workers identified using workplace surveillance demonstrates a clinically important rate of progression from BeS to CBD and to more clinically severe disease [17]. Mroz and colleagues showed that 19.3% of CBD cases who presented for clinical evaluation because of abnormal workplace surveillance BeLPTs developed clinical abnormalities requiring oral immunosuppressive therapy an average of 1.4 years after diagnosis [18]. At 30 years from first exposure, CBD patients had significantly more gas exchange impairment, manifest as higher resting and peak exercise alveolar-arterial gradient, lower rest and peak exercise arterial oxygen content, and lower diffusion capacity (DLCO) and total lung capacity. Over the course of the study, 8.8% of workers originally diagnosed as BeS developed CBD. Similarly, Duggal and colleagues found that after a mean of 7.4 years of follow-up, workers from a beryllium processing and production plant who had positive BeLPTs had average decreases in DLCO of 17.4 percentage points among both workers with BeS and CBD [19]. Whereas only one of 50 workers with BeS at baseline had abnormal lung function at follow-up, seven of 22 workers with biopsy proven CBD demonstrated abnormal lung function. Together, both studies demonstrate that workplace surveillance BeLPTs identify workers who have or progress to CBD, and that this disease, detected using the BeLPT in medical surveillance, is associated with significant functional impairment.

Updates on associations between BeLPT and exposure response—Cases of BeS continue to be detected through workplace BeLPT medical surveillance even in environments where exposure to beryllium is low, raising the questions as to whether a truly “safe” exposure level exists. In one beryllium processing facility where airborne Be levels were well below the 2.0 mcg/m^3 OSHA PEL, a baseline medical survey revealed 7% BeS and 4% CBD prevalences and estimated a sensitization incidence rate (IR) of 3.8/1000 person-months [20]. After implementing an enhanced multidisciplinary Be exposure prevention program including more frequent BeLPT surveillance, the sensitization IR decreased to 1.9/1000 person-months. Once a high-risk area was enclosed, the IR decreased further to 1.4/1000 person-months. Results from this study suggest that a multidisciplinary approach at exposure control, including more frequent BeLPT surveillance to identify areas of higher exposure and sensitization risk, is able to reduce sensitization in facilities even with low airborne concentrations of Be [21,22].

Mikulski and colleagues examined both Department of Energy (DOE) and Department of Defense (DOD) workers employed at a nuclear weapons facility where exposure largely occurred through machining and grinding of 1–2% beryllium copper-beryllium alloy tools [23,24].. Although only 6% of the DOE workers were considered to have higher exposure, 2.3% of them had BeS, (OR=4.58 compared to lowest exposure workers). Among the DOD workers, prevalence of BeS was 1.5%. The 2.3% and 1.5% sensitization rates in this “low

exposure" population, where the highest exposures were from refinishing copper-beryllium tools, were higher than expected. Similarly, Nilsen and colleagues found that 0.28% of employees of an aluminum smelter were sensitized to Be using the BeLPT [25]. In this workplace, the BeLPT was useful in identifying low rates of BeS in a population thought to have very low levels of Be exposure as an incidental presence in the processing of aluminum. Arjomandi and colleagues found a similar prevalence of BeS among current and former workers from Lawrence Livermore National Laboratory (LLNL), a nuclear weapons research and development facility [26]. The Be exposures at this facility were significantly lower than those at Rocky Flats, the most well-characterized DOE site, and well below 0.2 mcg/m^3. Nonetheless, the BeLPT detected cases of BeS among these low exposed workers, although the prevalence of CBD was five times lower than at Rocky Flats. Taken together, these studies support the notion that lower exposures are associated with lower rates of BeS and CBD.

Previous studies have shown that a glutamic acid (E) at amino acid position 69 (E69) of HLA-DPB1 Class II on APCs results in the more effective presentation of Be as an antigen, and increased risk of BeS and CBD in workers with this variant [27–37]. Van Dyke and colleagues studied associations between quantitative Be exposure and this genotype to better define exposure-related risk for BeS and CBD [38]. Whereas the E69 variant significantly increased risk for BeS, the development of CBD, but not BeS, was associated with higher Be exposure. Carriage of an E69 significantly increased odds of CBD, and each unit increase in lifetime weighted average Be exposure increased CBD odds twofold. This study showed that **both** increasing exposure and genetic susceptibility increased CBD risk, with no significant gene-by-environment interaction. Interestingly, after adjusting for E69 genetic risk factors, the study showed an exposure response relationship for Be exposure and CBD, but not for BeS. Cases of CBD were still detected even at extremely low Be levels, suggesting that a protective threshold exposure level has not yet been demonstrated.

BeLPT use in research to define therapies, understand disease progression, and develop new diagnostic modalities—Dobis and colleagues incorporated the BeLPT to demonstrate therapeutic effect in potential new treatments for CBD [39]. The authors tested the hypothesis that effective treatment of CBD patients with sulfasalazine or mesalamine, both antioxidants, could be measured by the inhibition of Be-stimulated peripheral blood mononuclear cell proliferation and cytokine production. They found that the BeLPT was significantly decreased in Be-stimulated CBD and BeS PBMCs treated with either drug. Based on these investigations an LPT may be used as the end point in the search for new investigational therapies.

In the quest to find non-invasive ways to diagnose CBD, Fireman and colleagues compared the ability of induced sputum (IS) CD4/CD8 ratio combined with positive blood BeLPTs against the use of biopsy to diagnose CBD [40]. They concluded that with the specificity and sensitivity of IS+ BeLPT+ reaching 92% and 100%, and excellent agreement between both methods (K=0.89, [0.74–1.0]), the use of IS CD4/CD8 ratio plus peripheral blood BeLPT may be an option for diagnosing CBD without need for bronchoscopy. Martin and colleagues studied current and former workers of a Be-machining facility to clarify the role of IFN-γ ELISPOT in diagnosing BeS and CBD [41]. Using a cut-point of 10 or more spot

forming units (SFU) of IFN-γ, ELISPOT yielded sensitivities and specificities of 85% and 100% for CBD, with PPV and NPV of 100% and 81%. IFN-γ ELISPOT was more sensitive in detecting BeS, as the test was positive in 10% of workers, compared to 4.2% measured by the BeLPT *(p<0.0001)*. ELISPOT was also useful in detecting CBD, as all 14 CBD subjects identified at the time of clinical evaluation had significantly increased production of IFN-γ detected on ELISPOT (27 SFU) compared with those BeS subjects who had not progressed. Advantages of the ELISPOT over the BeLPT to diagnosis sensitization include shorter duration of incubation, lack of radioactivity, and use of fetal bovine serum compared to human albumin [41]. Thus peripheral blood IFN-γ measurement via ELISPOT provides a potential new test that may provide additional data to the BeLPT or be able to identify additional workers with BeS. This may also help provide additional information regarding decisions on who should undergo biopsy to confirm CBD, thereby avoiding low-yield bronchoscopies, although additional study is needed.

Metal contact allergens

Occupationally-acquired dermal sensitization and allergic contact dermatitis from metals present opportunities for use of the LPT in better understanding underlying pathophysiologic mechanisms and in providing accurate and safer diagnostic methods.Martins and colleagues recently studied optimal conditions for performance of chromium LPT to detect allergy [42]. Those with chromium allergy, defined as dermatitis and positive patch tests, were compared to those without Cr dermatitis and negative patch tests. Six-day cultures yielded the best growth, and incubation with CrCl3 (Cr[III]) yielded the most consistent results. The most predictive LPT conditions for identifying allergic versus non-allergic individuals came from the use of nonfiltered Cr[III] solution, yielding sensitivity of 65%, specificity of 95% and accuracy of 80% in identifying dermatitis compared to presence of dermatitis with positive patch test.

Recent studies have compared the use of a tri- or hexavalent chromium LPT, chromium patch testing, and a chromium-specific ELISPOT in understanding occupational chromium allergy. Lindemann and colleagues studied whether each modality could identify chromium-sensitized individuals (CrS), and discriminate between those with and without clinical allergy [43]. Subjects with CrS and clinical allergy had significantly higher LPTs expressed as counts per minute compared to controls with no clinical allergy or positive patch tests, whereas CrS individuals without clinical allergy did not. Allergic subjects also had higher IFN-γ on ELISPOT testing than did controls. In this cohort, while the Cr LPT seemed to identify subjects with clinically-manifest allergy better than patch testing, positive LPT responses were not presented as values exceeding a stimulation threshold. These studies indicate that although the CrLPT may present a more accurate and safer diagnostic method to detect chromium allergy in patients with occupational exposure (because there is lower risk for inducing sensitization compared to patch testing), further refinements of testing methodologies are likely needed.

Updates on use of metal LPTs to evaluate surgical prosthetic implant allergy

—One of the recent advances in the use of LPT has arisen in the assessment of dysfunction of surgical prostheses. This field represents a new area of toxicologic assessment of metal

allergy with potential occupational surveillance applications and explores relationships with metal ions released as breakdown products into tissue and circulation.

Previously, most orthopedic implants were composed of nickel (Ni)-containing stainless steel, a metal associated with high rates of contact dermatitis. Currently, many are made of cobalt, chromium, and molybdenum alloys containing 0.5 to 1% nickel, or from a titanium/aluminum/vanadium alloy [44,45]. Several studies have demonstrated lymphocytic responses to metals in patients with loosened prosthetic joints, hypothesized due to hypersensitivity to wear-and-tear products of the prosthesis. Some patients have developed local skin reactions and pain in the tissue surrounding the prostheses, suggesting immunologically active antigens from the prosthesis itself [46].

One central question that the LPT may help elucidate among patients with failing surgical implants is whether hypersensitivity to different metals on LPT testing is associated with sensitization diagnosed on patch testing, and ultimately with graft dysfunction. Summer and colleagues found unique patterns of LPT and cytokine responses among subjects with and without implants, positive Ni patch tests, and implant dysfunction [47]. Those with Ni allergy and graft dysfunction showed strong lymphocyte proliferative responses and strong IL-17 expression, but virtually no IFN-gamma expression based on Ni-exposed PBMCs. Cobalt did not induce hypersensitivity or cytokine expression.

Some patients with metal-on-metal (MOM) implants following hip resurfacing arthropathy have developed peri-prosthetic pseudotumors, masses around the implant site that demonstrate lymphocytic infiltration on biopsy thought to be induced by a delayed type hypersensitivity reaction [45]. Most MOM implants are primarily composed of a Co-Cr-Mo alloy, with ≤ one percent Ni. Kwon and colleagues compared patients with pseudotumors and MOM Hip Resurfacing Arthroplasty (MOMHRA) to similar patients without pseudotumor, and to controls with no implants and no history of metal allergy. Subjects with MOMHRA had higher serum ion levels of Co and Cr compared to controls, but did not have positive LPTs to these metals, while those with pseudotumors had higher serum concentrations of Co and Cr compared to those with MOM implants only without pseudotumors. In contrast, some patients with MOMHRA had positive LPTs to nickel, but low serum concentrations of Ni. Notably, this study did not quantify metal ion levels in joint effusions or in the synovium, which may be more important.

If delayed-type hypersensitivity reactions to metals in implants does play a role in graft dysfunction, then pre-operative patch testing or LPT may identify individuals already sensitized and perhaps more likely to develop complications. Frigerio and colleagues found that self-reported history of metal allergy significantly under-identified metal-allergic patients compared to patch testing [44]. Metal LPTs added additional identification of subjects with sensitization, but the number of patients providing LPTs was too small to draw any conclusions from the study.

Non-orthopedic implants may serve as another risk for hypersensitivity. A systematic review examined hypersensitivity reactions to titanium in dental implants [48]. One article utilized an LPT in a case report of a woman who developed facial eczema after implantation of a

99.64% purity titanium implant. She had positive LPTs to TiCl3, NiSO4, and HgCl2. Her implant was removed, and her eczema resolved without medical treatment [49]. Another case report described the use of an LPT to the fibrin component of a glue used in arachnoid plasty to verify hypersensitivity as a complication in a patient undergoing neurosurgery [50]. In addition, case reports have identified Be as a source of gingivitis, although patch testing has usually been used clinically [51]. We are aware of cases of gingivitis related to Be-containing dental prostheses with demonstrated BeS using the BeLPT (Maier, personal communication). In summary, these reports suggest that further development of LPTs to surgical and dental implants may play increasingly important roles in understanding adverse immunologic outcomes, which may have collateral roles in the demonstration and knowledge of occupational allergy.

Use of novel LPT methodology in evaluation of occupational disease

Exposure to epoxy resin system chemicals has been associated with occupational asthma and allergic contact dermatitis [52,53]. Hines and colleagues found no significant differences in LPT positivity between epoxy-exposed versus unexposed manufacturing populations, despite significant differences in symptom reporting [54]. Two-part glues are also used extensively in orthopedic and dental procedures, including epoxy resins, cyanoacrylates, and methyl methacrylates. These are known potent sensitizers, although establishing the diagnosis remains difficult. LPT testing may provide an approach, although these tests are not yet clinically available.

Metalworking fluid (MWF) exposure is known to cause hypersensitivity pneumonitis (HP). While thought to represent a reaction to microbial contamination of the metalworking fluids, HP may also be related to sensitization from the MWF mixture itself. Anderson and colleagues studied three different types of MWFs and their ability to induce sensitization via LLNA [55]. All soluble and semi-synthetic MWFs induced notable increases in lymphocyte proliferation, whereas only one synthetic MWF induced proliferation. “TRIM VX,” the most irritating soluble MWF, is composed of oleic Acid and 4-chloro-3-methylphenol, which were sensitizers in LLNA assays. While worker surveillance LPTs were not utilized here, data from the LLNA studies suggests that MWFs themselves pose risks for sensitization. Development and use of an LPT in occupationally-exposed populations may be of benefit in risk assessment of MWF.

The healthcare field boasts high rates of occupational illnesses from various sensitizing agents. Ortho-phthalaldehyde (OPA) is replacing the use of glutaraldehyde as a sterilizing agent and is thought safer due to less volatility and no need for activation [6]. However, cases of anaphylaxis and occupational asthma have been reported in exposed workers [56]. Anderson and colleagues found a dose-dependent increase in draining lymph node proliferation and Th-2 cytokine response in LLNA based on expected workplace OPA concentrations [6]. Subsequently, Johnson and colleagues studied the potential for inhalation of OPA to cause respiratory sensitization in mice, demonstrating a concentration-dependent increase in total lymphocytes in draining lymph nodes [57]. Cytokine gene expression and lymphocyte phenotyping in the respiratory mucosa and draining lymph nodes of mice suggested that OPA can be a respiratory sensitizer. As OPA finds increasing use in

healthcare settings, an OPA LPT may play a role in medical surveillance of workers at increased risk for sensitization.

Parachlorobenzotrifluoride (PCBTF) is used in production of a wide range of products, but is primarily used as a solvent in commercial surface finishes [58–60]. Franko and colleagues found dose-dependent increases in draining lymph node proliferation in LLNA after treatment with PCBTF, along with increases in IFN-γ production [60]. This study demonstrated the sensitizing capacity of PCBTF in animal models at concentrations used in occupational settings. Worker populations who routinely use this solvent may benefit from avoidance of dermal contact and medical surveillance for sensitization by LPT.

Future tools for assessment of sensitization

The LPT is a highly specific tool to assess sensitization to particular occupational agents, although, it has limitations, including length of time needed to run the assay, exposure to radiation for workers performing the test and potential for variability in methodology [41]. Some investigators have examined flow cytology methods to assess lymphocyte proliferative responses to beryllium. *The carboxyfluorescein diacetate succinimidyl ester* (CFSE) labeling method identifies beryllium-specific proliferative responses and immunophenotyping without the need for radioactive agents, while using a single time point, with the ability to be run on commercial clinical flow cytometers [61]. Further study on CFSE-based flow cytometry methods may identify a safer and more efficient tool to diagnose BeS. Similarly, the studies above suggest that ELISPOT may also provide an alternative or adjuvant to BeLPT testing [41, 43].

Summary

The LPT currently has validated uses in the assessment of occupationally-acquired sensitization to Be, and holds promise for the assessment of sensitization to other contact allergens and emerging sensitizers such as metalworking fluids, hospital sterilizing agents and solvents. The well-standardized methodology of the BeLPT model permits extension to other agents to define sensitization and disease. As noted in the case of beryllium, LPTs may help us understand the natural history of sensitization and disease, define exposure-disease response relationships, and develop new therapies and new diagnostic modalities. The LLNA may find similar application in identifying agents as potential sensitizers that will require additional evaluation and testing with the LPT. Finally, future generation of tests may allow detection of hypersensitivity and determining the type of hypersensitivity response by using safer and more efficient methods.

Acknowledgments

Conflicts of interest:

Drs. Maier and Pacheco evaluate patients clinically using the BeLPT and other LPTs and interpret the tests, and NJH provides this testing commercially for these and other patients. In addition, Dr. Maier has received NIH grant funding to evaluate the immune response to beryllium, and the impact of potential therapeutics on this response and the BeLPT. Dr. Hines received NIH and CDC-NIOSH pilot grand funding to study epoxy resin LPT responses.

References

1. Samuel G, Maier LA. Immunology of chronic beryllium disease. Curr Opin Allergy Clin Immunol. 2008; 8:126–34. [PubMed: 18317020]
2. Department of Energy (US). Chronic Beryllium Disease Prevention Program, Final Rule. Code of Federal Regulations. 1999 Title 10, vol 64, part 850, subpart 23.
3. Frome EL, Newman LS, Cragle DL, Colyer SP, Wambach PF. Identification of an abnormal beryllium lymphocyte proliferation test. Toxicology. 2003 Feb 1; 183(1–3):39–56. [PubMed: 12504341]
4. Wambuch, P. Beryllium lymphocyte proliferation testing (BeLPT). Document Number DOE-SPEC-1142-2001. Springfield, VA 22161: National Technical Information Service; 2001.
5. Basketter DA. Skin sensitization: strategies for the assessment and management of risk. Br J Dermatol. 2008 Aug; 159(2):267–73. [PubMed: 18503602]

6*. Anderson SE, Umbright C, Sellamuthu R, Fluharty K, Kashon M, Franko J, et al. Irritancy and allergic responses induced by topical application of ortho-phthalaldehyde. Toxicol Sci. 2010 Jun; 115(2):435–43. This article shows that an emerging popular substitute for the sensitizer glutaraldehyde, ortho-phthalaldehyde, shows clear evidence for sensitizing potential in local lymph node assay. Workers exposed to this agent might benefit from medical surveillance for sensitization via LPT. [PubMed: 20176622]

7. (NIEHS) National Institute of Environmental Health Science. The murine local lymph node assay: a test method for assessing the allergic contact dermatitis potential of chemicals/compounds. Fed Regist. 1999; 64:14006–7. [PubMed: 10558409]
8. Kreiss K, Mroz MM, Zhen B, Martyny JW, Newman LS. Epidemiology of beryllium sensitization and disease in nuclear workers. Am Rev Respir Dis. 1993 Oct; 148(4 Pt 1):985–91. [PubMed: 8214955]
9. Kreiss K, Wasserman S, Mroz MM, Newman LS. Beryllium disease screening in the ceramics industry. Blood lymphocyte test performance and exposure-disease relations. J Occup Med. 1993 Mar; 35(3):267–74. [PubMed: 8455096]
10. Mroz MM, Kreiss K, Lezotte DC, Campbell PA, Newman LS. Reexamination of the blood lymphocyte transformation test in the diagnosis of chronic beryllium disease. J Allergy Clin Immunol. 1991 Jul; 88(1):54–60. [PubMed: 2071785]
11. Kreiss K, Newman LS, Mroz MM, Campbell PA. Screening blood test identifies subclinical beryllium disease. J Occup Med. 1989 Jul; 31(7):603–8. [PubMed: 2788726]
12. Kreiss K, Newman LS, Mroz M. Blood testing for chronic beryllium disease. J Occup Med. 1991 Nov; 33(11):1188–9. [PubMed: 1765863]
13. Stange AW, Furman FJ, Hilmas DE. The beryllium lymphocyte proliferation test: Relevant issues in beryllium health surveillance. Am J Ind Med. 2004 Nov; 46(5):453–62. [PubMed: 15490468]
14. Middleton DC, Fink J, Kowalski PJ, Lewin MD, Sinks T. Optimizing BeLPT criteria for beryllium sensitization. Am J Ind Med. 2008 Mar; 51(3):166–72. [PubMed: 18181198]
15. Middleton DC, Lewin MD, Kowalski PJ, Cox SS, Kleinbaum D. The BeLPT: algorithms and implications. Am J Ind Med. 2006 Jan; 49(1):36–44. [PubMed: 16362939]

16**. Middleton DC, Mayer AS, Lewin MD, Mroz MM, Maier LA. Interpreting borderline BeLPT results. Am J Ind Med. 2011 Mar; 54(3):205–9. Based on current testing algorthims, workers undergoing surveillance BeLPT, this study demonstrated the value of borderline BeLPT results in confirming BeS. At 2% prevalence, post-test probabilities for 3-result combinations including borderline tests ranged from 72% to 100%, with likelihood increasing as prevalence increases. This suggests that borderline results truly do have value in diagnosing BeS and potentially CBD. [PubMed: 20957676]

17. Newman LS, Mroz MM, Balkissoon R, Maier LA. Beryllium sensitization progresses to chronic beryllium disease: a longitudinal study of disease risk. Am J Respir Crit Care Med. 2005 Jan 1; 171(1):54–60. [PubMed: 15374840]

18**. Mroz MM, Maier LA, Strand M, Silviera L, Newman LS. Beryllium lymphocyte proliferation test surveillance identifies clinically significant beryllium disease. Am J Ind Med. 2009 Oct; 52(10):762–73. This study evaluated all patients who were found to have CBD upon clinical

evaluation at National Jewish Health after referral following positive workplace surveillance BeLPT. They found that 19.3% of CBD cases required use of immunosuppressive therapy an average of 1.4 years after initial diagnosis, and that they had significantly lower resting and exercise arterial oxygen content and higher A-a gradient than did BeS patients. This study supports that CBD detected via initial workplace BeLPT surveillance identifies workers with clinically significant disease. [PubMed: 19681064]

19**. Duggal M, Deubner DC, Curtis AM, Cullen MR. Long-term follow-up of beryllium sensitized workers from a single employer. BMC Public Health. 2010; 10:5. This study challenged the hypothesis that CBD detected via workplace surveillance using LPT is not severe. They found that workers with positive BeLPT had significantly lower diffusion capacity by 17.4% of predicted on pulmonary function testing. One third of those found to have CBD at initial exam developed clinical findings suggestive of CBD by the time of follow-up. This tends to suggest that workers identified as having BeS and CBD on workplace surveillance do indeed have clinically relevant disease. [PubMed: 20047684]

20**. Thomas CA, Bailey RL, Kent MS, Deubner DC, Kreiss K, Schuler CR. Efficacy of a program to prevent beryllium sensitization among new employees at a copper-beryllium alloy processing facility. Public Health Rep. 2009 Jul-Aug;124(Suppl 1):112–24. A multidisciplinary exposure control program was instituted at a beryllium processing facility that included enhanced engineering and administrative controls, including more frequent BeLPT testing. Despite most exposures being below the OSHA exposure limit, use of more frequent BeLPT surveillance was an important factor in reducing the number of BeS cases detected, demonstrating the utility of the BeLPT as part of a disease prevention program. [PubMed: 19618813]

21. Cummings KJ, Stefaniak AB, Virji MA, Kreiss K. A reconsideration of acute Beryllium disease. Environ Health Perspect. 2009 Aug; 117(8):1250–6. [PubMed: 19672405]

22. Bailey RL, Thomas CA, Deubner DC, Kent MS, Kreiss K, Schuler CR. Evaluation of a preventive program to reduce sensitization at a beryllium metal, oxide, and alloy production plant. J Occup Environ Med. 2010 May; 52(5):505–12. [PubMed: 20431418]

23*. Mikulski MA, Leonard SA, Sanderson WT, Hartley PG, Sprince NL, Fuortes LJ. Risk of beryllium sensitization in a low-exposed former nuclear weapons cohort from the Cold War era. Am J Ind Med. 2011 Mar; 54(3):194–204. Both Mikulski studies evaluated populations of beryllium-exposed workers from a nuclear weapons facility in operation during the Cold War. The only exposures sustained would have been in the machining or grinding in refinishing of 2% beryllium copper-beryllium parts, a work practice where exposures were thought to be low. BeLPT was useful in identifying cases of BeS in this population where risk of BeS and CBD was thought low, as no pure beryllium processing occurred there. [PubMed: 21298695]

24*. Mikulski MA, Sanderson WT, Leonard SA, Lourens S, Field RW, Sprince NL, et al. Prevalence of beryllium sensitization among Department of Defense conventional munitions workers at low risk for exposure. J Occup Environ Med. 2011 Mar; 53(3):258–65. Both Mikulski studies evaluated populations of beryllium-exposed workers from a nuclear weapons facility in operation during the Cold War. The only exposures sustained would have been in the machining or grinding in refinishing of 2% beryllium copper-beryllium parts, a work practice where exposures were thought to be low. BeLPT was useful in identifying cases of BeS in this population where risk of BeS and CBD was thought low, as no pure beryllium processing occurred there. [PubMed: 21293302]

25*. Nilsen AM, Vik R, Behrens C, Drablos PA, Espevik T. Beryllium sensitivity among workers at a Norwegian aluminum smelter. Am J Ind Med. 2010 Jul; 53(7):724–32. Cases of BeS were identified via use of BeLPT among workers at an aluminum smelter, where the only exposure to beryllium occurred through trace levels of Be present in aluminum. BeLPT may be helpful even in environments not traditionally thought to be at risk for beryllium exposure. [PubMed: 20187010]

26**. Arjomandi M, Seward J, Gotway MB, Nishimura S, Fulton GP, Thundiyil J, et al. Low prevalence of chronic beryllium disease among workers at a nuclear weapons research and development facility. J Occup Environ Med. 2010 Jun; 52(6):647–52. This study found similar prevalence of BeS at Lawrence Livermore National Laboratory as at Rocky Flats nuclear weapons production facility, despite the exposure levels at the former being significantly lower than those at the latter. Prevalence of CBD however, was much lower at Lawrence Livermore

National Laboratory. This study calls into question whether an exposure-response gradient or threshold exposure level may be present for CBD but not for BeS. [PubMed: 20523233]

27. Richeldi L, Kreiss K, Mroz MM, Zhen B, Tartoni P, Saltini C. Interaction of genetic and exposure factors in the prevalence of berylliosis. Am J Ind Med. 1997 Oct; 32(4):337–40. [PubMed: 9258386]
28. Richeldi L, Sorrentino R, Saltini C. HLA-DPB1 glutamate 69: a genetic marker of beryllium disease. Science. 1993 Oct 8; 262(5131):242–4. [PubMed: 8105536]
29. Saltini C, Richeldi L, Losi M, Amicosante M, Voorter C, van den Berg-Loonen E, et al. Major histocompatibility locus genetic markers of beryllium sensitization and disease. Eur Respir J. 2001 Oct; 18(4):677–84. [PubMed: 11716174]
30. Wang Z, Farris GM, Newman LS, Shou Y, Maier LA, Smith HN, et al. Beryllium sensitivity is linked to HLA-DP genotype. Toxicology. 2001 Aug 13; 165(1):27–38. [PubMed: 11551429]
31. Wang Z, White PS, Petrovic M, Tatum OL, Newman LS, Maier LA, et al. Differential susceptibilities to chronic beryllium disease contributed by different Glu69 HLA-DPB1 and -DPA1 alleles. J Immunol. 1999 Aug 1; 163(3):1647–53. [PubMed: 10415070]
32. Rossman MD, Stubbs J, Lee CW, Argyris E, Magira E, Monos D. Human leukocyte antigen Class II amino acid epitopes: susceptibility and progression markers for beryllium hypersensitivity. Am J Respir Crit Care Med. 2002 Mar 15; 165(6):788–94. [PubMed: 11897645]
33. Maier LA, McGrath DS, Sato H, Lympany P, Welsh K, Du Bois R, et al. Influence of MHC class II in susceptibility to beryllium sensitization and chronic beryllium disease. J Immunol. 2003 Dec 15; 171(12):6910–8. [PubMed: 14662898]
34. McCanlies EC, Ensey JS, Schuler CR, Kreiss K, Weston A. The association between HLA-DPB1Glu69 and chronic beryllium disease and beryllium sensitization. Am J Ind Med. 2004 Aug; 46(2):95–103. [PubMed: 15273960]
35. Snyder JA, Demchuk E, McCanlies EC, Schuler CR, Kreiss K, Andrew ME, et al. Impact of negatively charged patches on the surface of MHC class II antigen-presenting proteins on risk of chronic beryllium disease. J R Soc Interface. 2008 Jul 6; 5(24):749–58. [PubMed: 17956852]
36. Gaede KI, Amicosante M, Schurmann M, Fireman E, Saltini C, Muller-Quernheim J. Function associated transforming growth factor-beta gene polymorphism in chronic beryllium disease. J Mol Med (Berl). 2005 May; 83(5):397–405. [PubMed: 15750822]
37. Snyder JA, Weston A, Tinkle SS, Demchuk E. Electrostatic potential on human leukocyte antigen: implications for putative mechanism of chronic beryllium disease. Environ Health Perspect. 2003 Nov; 111(15):1827–34. [PubMed: 14630515]

38**. Van Dyke MV, Martyny JW, Mroz MM, Silveira LJ, Strand M, Fingerlin TE, et al. Risk of chronic beryllium disease by HLA-DPB1 E69 genotype and beryllium exposure in nuclear workers. Am J Respir Crit Care Med. 2011 Jun 15; 183(12):1680–8. This study found two key findings. First, there was a exposure-response gradient seen for CBD using quantitative Be exposure data. Second, there was also an independent effect of genetic variants at the E69 allele increasing risk for both BeS and CBD, with no significant gene-by-environment interaction. This is a key finding given previous work suggesting that risk for CBD likely is mediated by gene-by-environment interactions. [PubMed: 21471109]

39**. Dobis DR, Sawyer RT, Gillespie MM, Newman LS, Maier LA, Day BJ. Sulfasalazine and mesalamine modulate beryllium-specific lymphocyte proliferation and inflammatory cytokine production. Am J Respir Cell Mol Biol. 2010 Oct; 43(4):458–64. This is the first study to use the BeLPT as a tool to assess new medical treatments for CBD. Mesalamine and sulfasalzine were able to diminish lymphocyte proliferation in stimulated cells, suggesting a potential role for treatment. [PubMed: 19901345]

40*. Fireman E, Mazor O, Kramer M, Priel I, Lerman Y. Non-invasive diagnosis of chronic beryllium disease in workers exposed to hazardous dust in Israel. Occup Environ Med. 2010 Sep; 67(9): 631–5. While current algorithms for diagnosis of CBD depend on semi-invasive techniques such as bronchoscopy, this study demonstrated that induced sputum CD4/CD8 ratio coupled with peripheral blood BeLPT could diagnose CBD with high sensitivity and specificity. [PubMed: 19955573]

41**. Martin AK, Mack DG, Falta MT, Mroz MM, Newman LS, Maier LA, et al. Beryllium-specific CD4(+) T cells in blood as a biomarker of disease progression. J Allergy Clin Immunol. 2011

Nov; 128(5):1100–6. e5. Compared to BeLPT, use of an IFN-γ ELISPOT on blood from workers of a Be-machining facility identified more cases of sensitization, and was useful in distinguishing those who would go on to develop CBD compared to non-progressors. This study identifies a potential new avenue for testing for BeS and CBD that may have signficiant technical and safety advantages compared to the BeLPT. [PubMed: 21943943]

42*. Martins LE, Duarte AJ, Aoki V, Nunes RS, Ogusuku S, Reis VM. Lymphocyte proliferation testing in chromium allergic contact dermatitis. Clin Exp Dermatol. 2008 Jul; 33(4):472–7. Various concentrations and formulations of chromium were studied to define optimal conditions to perform chromium LPT. With further work, chromium LPT could become a useful tool in occupational surveillance. [PubMed: 18582233]

43**. Lindemann M, Rietschel F, Zabel M, Grosse-Wilde H. Detection of chromium allergy by cellular in vitro methods. Clin Exp Allergy. 2008 Sep; 38(9):1468–75. Sensitivity to chromium detected as total counts per minute on LPT was higher in subjects with chromium allergy and positive patch tests compared to controls, but not when expressed as Stimulation Index. This study correlated positive patch tests results to LPT and to IFN-γ ELISPOT in diagnosing chromium allergy. Further methodologic development is likely needed before chromium LPT may be preferred to patch testing to diagnose chromium allergy. [PubMed: 18384428]

44*. Frigerio E, Pigatto PD, Guzzi G, Altomare G. Metal sensitivity in patients with orthopaedic implants: a prospective study. Contact Dermatitis. 2011 May; 64(5):273–9. These authors found that subjective history of "allergy" to a specific metal did not correlate with patch test findings; thus, suggesting that some objective measure of sensitization prior to placement of metal orthopedic implants is superior to clinical history. However, this study could not provide substantial evidence to suggest whether LPT could be superior to patch testing. [PubMed: 21480913]

45**. Kwon YM, Thomas P, Summer B, Pandit H, Taylor A, Beard D, et al. Lymphocyte proliferation responses in patients with pseudotumors following metal-on-metal hip resurfacing arthroplasty. J Orthop Res. 2010 Apr; 28(4):444–50. Interesting study exploring the issue of metal hypersensitivity as a mechanism for the development of pseudotumors in patients with metal-on-metal hip implants. Patients with pseudotumors had positive LPTs to nickel, but not to cobalt or chromium, all of which were present in the implants. Notably, serum ion concentrations of the metals, surrogates for joint loosening via wear-and-tear, were inversely proportional to LPT positivity. This is a key study in exploring relationships between orthopedic implant failure and underlying immunologic pathophysiologic mechanism. [PubMed: 19834954]

46. Revell PA. The combined role of wear particles, macrophages and lymphocytes in the loosening of total joint prostheses. J R Soc Interface. 2008 Nov 6; 5(28):1263–78. [PubMed: 18647740]

47*. Summer B, Paul C, Mazoochian F, Rau C, Thomsen M, Banke I, et al. Nickel (Ni) allergic patients with complications to Ni containing joint replacement show preferential IL-17 type reactivity to Ni. Contact Dermatitis. 2010 Jul; 63(1):15–22. This study found positive nickel LPTs and higher levels of IFN-γ in patients with failing orthopedic implants, but no responses to cobalt. This study supports the concept of sensitization to nickel as a potential pathogenic mechanism leading to early implant failure, and demonstrates utility of LPT in diagnosis. [PubMed: 20597929]

48. Javed F, Al-Hezaimi K, Almas K, Romanos GE. Is Titanium Sensitivity Associated with Allergic Reactions in Patients with Dental Implants? A Systematic Review. Clin Implant Dent Relat Res. 2011 Mar 17.

49. Egusa H, Ko N, Shimazu T, Yatani H. Suspected association of an allergic reaction with titanium dental implants: a clinical report. J Prosthet Dent. 2008 Nov; 100(5):344–7. [PubMed: 18992567]

50. Kanazawa R, Sato S, Iwamoto N, Teramoto A. Allergic reaction following arachnoid plasty with a fibrin sealant. Neurol Med Chir (Tokyo). 2010; 50(7):608–10. [PubMed: 20671393]

51. Toledo F, Silvestre JF, Cuesta L, Latorre N, Monteagudo A. Contact allergy to beryllium chloride: report of 12 cases. Contact Dermatitis. 2011 Feb; 64(2):104–9. [PubMed: 21210824]

52. Bray PG. Epoxy resins. Occup Med. 1999 Oct-Dec;14(4):743–58. [PubMed: 10495483]

53. Scherpereel A, Tillie-Leblond I, Pommier de Santi P, Tonnel AB. Exposure to methyl methacrylate and hypersensitivity pneumonitis in dental technicians. Allergy. 2004 Aug; 59(8):890–2.

54. Hines SE, Barker L, Nagabhushanam V, Robinson M, Duvall K, Gaitens J, et al. Respiratory symptoms, spirometry and immunologic sensitivity in epoxy resin workers. Am J Respir Crit Care Med. 2011; 183:A1179.

55**. Anderson SE, Brown KK, Butterworth LF, Fedorowicz A, Jackson LG, Frasch HF, et al. Evaluation of irritancy and sensitization potential of metalworking fluid mixtures and components. J Immunotoxicol. 2009 Mar; 6(1):19–29. Current thinking behind the etiology of hypersensitivity pneumonitis from metalworking fluids is related to microbial contamination of the fluids; however, this study suggests that the metalworking fluids themselves have sensitization potential. Use of the LPT in medical surveillance of workers exposed to metalworking fluids may be of benefit. [PubMed: 19519159]

56. Sokol WN. Nine episodes of anaphylaxis following cystoscopy caused by Cidex OPA (ortho-phthalaldehyde) high-level disinfectant in 4 patients after cytoscopy. J Allergy Clin Immunol. 2004 Aug; 114(2):392–7. [PubMed: 15316522]

57*. Johnson VJ, Reynolds JS, Wang W, Fluharty K, Yucesoy B. Inhalation of ortho-phthalaldehyde vapor causes respiratory sensitization in mice. J Allergy (Cairo). 2011; 2011:751052. After demonstrating sensitization potential of this glutaraldehyde replacement via local lymph node assay, NIOSH researchers demonstrated potential for respiratory sensitization in mice following inhalational challenge. Use of the LPT in medical surveillance of workers exposed to ortho-phthalaldehyde may be of benefit. [PubMed: 21785612]

58. NTP National Toxicology Program. Chemical Information Profile for 1-Chloro-4-(trifluoromethyl)benzene. Research Triangle Park, NC, USA: National Institute of Environmental Health Sciences; 2009.

59. Maul JJ, Ostrowski PJ, Ublacker GA, Linclau B, Curran DP. Benzotrifluoride and derivatives: Useful solvents for organic synthesis and fluorous synthesis. Top Curr Chem. 1999; 206:79–105.

60*. Franko J, Jackson LG, Meade BJ, Anderson SE. Allergic Potential and Immunotoxicity Induced by Topical Application of 1-Chloro-4-(Trifluoromethyl)Benzene (PCBTF) in a Murine Model. J Allergy (Cairo). 2011; 2011:238513. Both lymphocyte proliferation via local lymph node assay and IFN-γ production in mice exposed to the chemical PCBTF suggest that the agent is a sensitizer. PCBTF is used in many different industrial applications, therefore yielding many opportunities for occupational exposure, where LPT may be of benefit via surveillance. [PubMed: 21747864]

61. Milovanova TN, Popma SH, Cherian S, Moore JS, Rossman MD. Flow cytometric test for beryllium sensitivity. Cytometry B Clin Cytom. 2004 Jul; 60(1):23–30. [PubMed: 15221866]

Key points

- LPT continues to be useful in occupational medical surveillance for beryllium-exposed workers at risk not only for sensitization, but for clinically significant disease with functional impairment and need for immunosuppressive medication.
- LPT results may correlate with quantitative exposure assessment and contribute to our knowledge of dose-response relationships, threshold levels for health effects, and genetic predisposition to disease in beryllium-exposed workers.
- LPT use may help understand unusual conditions like surgical implant dysfunction, that may be a manifestation of hypersensitivity reactions to sensitizing metals and adhesives, with parallel correlations to occupational contact allergy.